A FACILE METHOD OF REGULATING THE DYNAMIC RANGE OF L-LACTATE SENSOR BASED ON POLYMER COATING

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The enzyme layer of L-lactate oxidase (LOD)-modified platinum electrode (L-lactate sensor) was covered with polymer membrane to regulate the dynamic range of the sensor. The upper detection limit of the sensor was significantly improved by the use of acetyl cellulose membrane.

KEYWORDS L-lactate sensor; L-lactate oxidase; acetyl cellulose membrane; dynamic range

The development of electrochemical sensors for clinical use has been a focal subject in analytical science and technology.[1] Several authors have reported amperometric L-lactate sensors by the use of L-lactate dehydrogenase[2,3] or L-lactate oxidase.[4,5] The electrochemical response of the sensors is highly sensitive to L-lactate, the lower limit of detection being typically at a micromolar level of L-lactate or less. On the other hand, the upper limit of detection of these sensors is often insufficient for the determination of L-lactate in human blood because of the rather high level of L-lactate in blood. For example, in the case of lactate acidosis, L-lactate is accumulated in blood to the level of 10 mM or more. For this reason, it is important to improve the upper detection limit of L-lactate sensor. The present communication reports a facile method of regulating the dynamic range of L-lactate sensor based on polymer coating.

A platinized platinum wire electrode (diameter:0,1 mm, effective length:1,0 mm) was coated with a bovine serum albumin (BSA) membrane which was prepared by electrochemical deposition according to the reported procedure.[2] The BSA layer was further modified with L-lactate oxidase (LOD) by immersing the BSA-coated probe in 25% glutaraldehyde solution for 20 min and then in 5% LOD solution (phosphate buffer, pH 7.4) for 20 min. Thus, the LOD-modified platinum electrode (L-lactate sensor) was fabricated. The acetyl cellulose membrane was coated on the enzyme layer of the electrode by dip-coating using 1% or 2% acetyl cellulose solution in acetone. The acetyl cellulose used was a commercial product in which ca. 48% of the hydroxy group was acetylated. The L-lactate sensor thus prepared was used as the working electrode in a three-electrode system together with counter (platinum wire) and reference (Ag/AgCl) electrodes. The experimental setup for the measurement of sensor response is illustrated in Fig. 1.

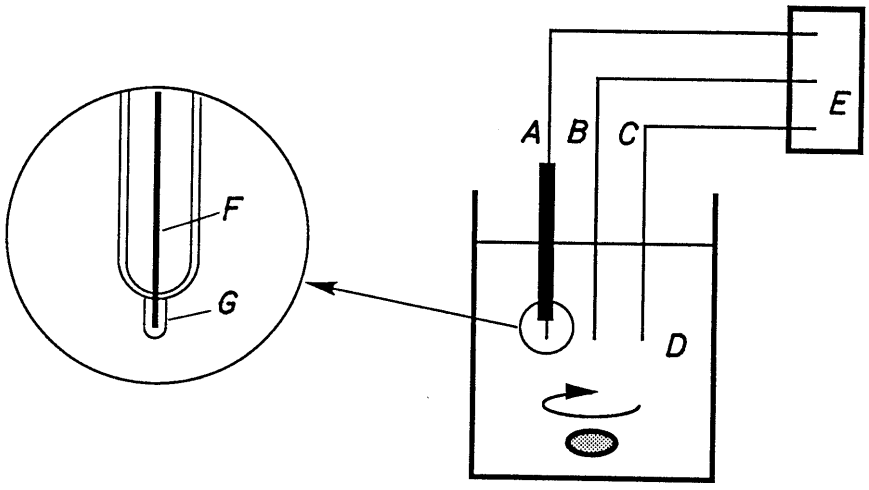

Fig. 1. Experimental Setup for the Measurement of Sensor Response
(A)LOD-modified electrode, (B)counter electrode, (C)reference electrode, (D)L-lactate solution, (E)potentiostat, (F)platinum wire, and (G)LOD layer.

Figure 2 shows typical response curves of the sensors with and without acetyl cellulose membrane. The sensors showed amperometric response to L-lactate at the potential of +0.6 V vs. Ag/AgCl. The response of the sensor arises from the electrochemical oxidation of H_2O_2 which is produced by the enzymatic reaction of L-lactate as follows:

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + H_2O_2$$

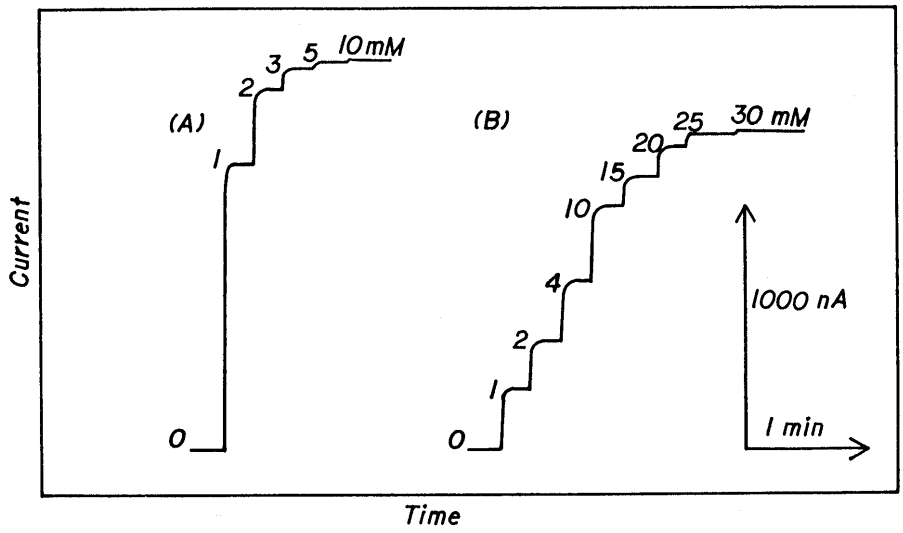

Fig. 2. Typical Response Curves of L-Lactate Sensors with (B) and without (A) Acetyl Cellulose Membrane
2% acetyl cellulose solution was used to prepare the polymer coating.

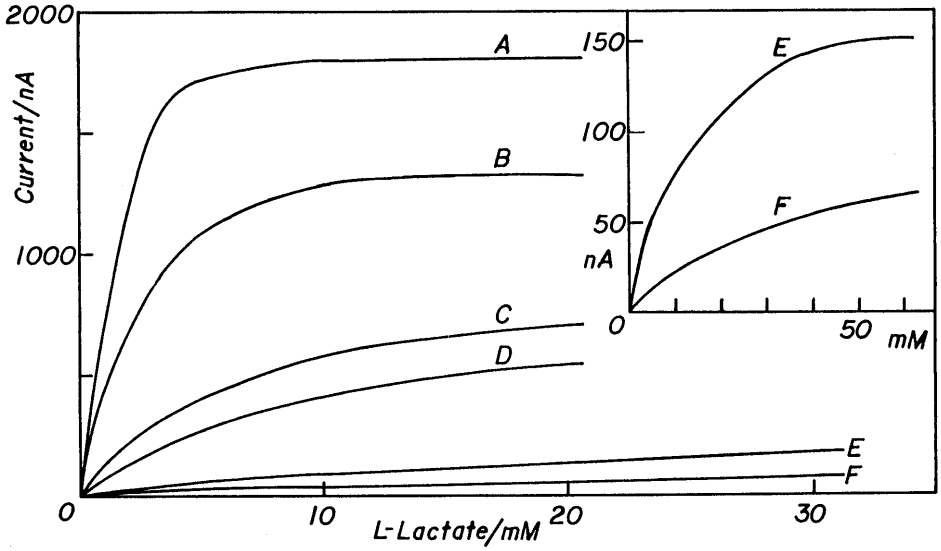

Fig. 3. Effects of the Thickness of Polymer Coating on the Calibration Graph of the L-Lactate Sensor
Calibration graphs of the sensors without coating (A) and with single (B), 2-fold (C), 3-fold (D), 5-fold (E) and 10-fold coatings (F) are shown. 1% acetyl cellulose solution was used to prepare the polymer coating.

The response time of the sensor is very fast (within 10 s), and the effect of polymer coating on the response time is small. It should be noted that the dynamic range of the sensor is modified by the polymer coating. The polymer-coated sensor showed response to L-lactate up to 20 mM , while the response of the bare sensor declined around 3-4 mM L-lactate. These results strongly suggest that the acetyl cellulose membrane can be used to regulate the dynamic range of the sensor.

Figure 3 shows the calibration graphs of the L-lactate sensors coated with acetyl cellulose membranes of varying thickness. The thickness of the membrane was increased successively by repeating the dip-coating process. It is clear that the dynamic range of the sensor is extended by the polymer coating, depending on the thickness of the membrane. The magnitude of the output signal decreased with increases membrane thickness. For example, the output current was suppressed to lower than 100 nA for the sensor with 10-fold coating. However, this is not a crucial drawback because the electric signal in nA level can be detected without any difficulty under the present experimental conditions. The response time of the sensor with a thicker membrane was slightly longer than that of the membrane-free sensor, being typically 10-20 sec for the sensor with 5-fold or 10-fold coating as compared with 10 sec or less for the bare sensor. The electrochemical response was fairly reproducible, and the sensors could be used repeatedly for more than one month. These results imply that the acetyl cellulose membrane serves as a barrier for L-lactate to diffuse from the sample solution to the enzyme layer. We checked the effect of acetone on the sensor response, because the acetyl cellulose membrane was coated from the acetone solution. The catalytic activity of LOD of the sensor was little affected by exposure of the LOD layer to acetone for a short time. This means that the modification in the sensor response is not originating from the deactivation of LOD by exposure to acetone. Thus, we have demonstrated that the dynamic range of the L-lactate sensor can be controlled by regulating the thickness of the acetyl cellulose membrane. The present technique is more facile and inexpensive than the alternative technique in which the loading of enzyme is adjusted appropriately. Another merit of this technique is that the enzyme layer of the sensor may be protected from the possible adsorption of proteins dissolved in samples such as blood.

Other polymers such as poly(vinyl alcohol) (PVA), poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(vinylpyrrolidone-vinyl acetate) (PVP-VA) were also used as material for the polymer coating. The LOD-layer of the sensor was coated similarly with these polymers, which were dissolved in water (PVA), ethanol (PHEMA), or dichloromethane (PVA-VA). These polymers, unfortunately, did not improve the dynamic range of the sensor.

Studies are now in progress to elucidate the optimum conditions for preparing the acetyl cellulose membrane and to identify more useful materials for the polymer coating.

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